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Vira Fedorchenko [*,†], Maiia Ananieva [†], Galina Loban' [†], Tetyana Ivakhnyuk [†], Natalia Hasiuk [†], Nellia Bobrova [†]

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Sphingomonas paucimobilis in Children with Recurrent Tonsillitis

Vira Fedorchenko [1,*,†], Maiia Ananieva [1,2,†], Galina Loban' [1,†], Tetyana Ivakhnyuk [3,†], Natalia Hasiuk [4,†] and Nellia Bobrova [1,†]

[1] Department of Microbiology, Virology and Immunology, Poltava State Medical University, Poltava
[2] Centralised bacteriological laboratory. Municipal enterprise "3-rd city clinical hospital of Poltava city council", Poltava, Ukraine
[3] Department of Community Health, Sumy State University Medical Institute, Sumy, Ukraine
[4] Department of Paediatrics No. 1 with Neonatology, Poltava State Medical University, Poltava, Ukraine
* Correspondence: v.fedorchenko@pdmu.edu.ua; +380665070759
† These authors contributed equally to this work.

Abstract

Background: *S. paucimobilis* is a Gram-negative bacterium widely distributed in natural environments, hospitals, and highly resistant to disinfectants. Various infections in humans have been reported as sporadic case reports. This study aimed to quantify the presence of *S. paucimobilis* in the fauces microbial landscape of children with recurrent tonsillitis and the sensitivity of isolates to antimicrobials. **Methods:** The bacteriological method of research was used. The final identification was performed on Vitec-2 compact bioMarieux automatic bacteriological analyser (France). Antimicrobial susceptibility was determined using the measurement of the inhibition zone diameters (EUCAST standardised disk diffusion methodology) on the Mueller-Hinton (MH) agar. **Results:** Three isolates *of S. paucimobilis* were isolated from nasopharyngeal material from five children. The growth of *S. paucimobilis* was observed in three sectors of Columbia agar with 5% sheep blood (bioMarieux, France), as well as other bacterial species that were most abundant. The cultures were variably sensitive to most of the antibiotics used and resistant to Aztreonam. **Conclusions:** Human beings, as well as environmental objects, are likely to be a source of *S. paucimobilis*. Among the antimicrobials of choice for empirical antibiotic therapy, the best ones are the clavulanic acid-protected β-lactam antibiotics, Piperacillin Tazobactam, Carbopenems, and Rifampicin.

Keywords: fauces; antroponosis; sapronosis; antimicrobials; nosocomial infection; epidemiology

1. Introduction

It is currently known that *S. paucimobilis* is a glucose-no fermenting Gram-negative bacterium that is widely distributed in both the natural environment and hospitals, an important characteristic of the microorganism for medicine is its high resistance to disinfectants [1–3]. The high ability of bacteria of the genus *Sphingomonas* to biodegrade organic compounds is known [4,5], and the possibilities of using *S. paucimobilis* for biomedical and biotechnological purposes are being investigated. Various infections caused by this bacterium in humans have been reported. Most of the reports have been limited to sporadic case reports [6].

Thus, *S. paucimobilis* are present in various environments surrounding humans, which causes constant human contact with these microorganisms, so their potential role in the development of human infectious pathology deserves further study.

This study aims to quantify the presence of *S. paucimobilis* in the fauces microbial landscape of children with recurrent tonsillitis and the sensitivity of isolates to antimicrobials. We specifically investigated how often *S. paucimobilis* was present in the material obtained from the mucosal membrane of fauces of the children with recurrent tonsillitis and the level of the microbial

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colonisation of the investigated area by this microorganism. Our findings contributed to more nuanced understanding of the epidemiology of infections caused by this microorganism, and also what antimicrobials can be used for the empirical antibiotic therapy.

The principal conclusions: *S. paucimobilis* can be transmitted from patients to the environment. Human beings, as well as environmental objects, are likely to be a source of *S. paucimobilis*. The clavulanic acid-protected β-lactam antibiotics, Piperacillin Tazobactam and Carbopenems, and Rifampicin are the drugs of choice for empirical antibiotic therapy.

2. Materials and Methods

Investigation was conducted at the Department of Paediatrics No 1 with Neonatology, Poltava State Medical University, Poltava, Ukraine, and Department of Microbiology, Virology and Immunology, Poltava State Medical University, Poltava, Ukraine. All samples tested for determination of qualitative and quantitative characteristics of the composition of microorganisms in pathological material obtained from the fauces of children with recurrent tonsillitis in the acute stage. The samples collected from 25 February 2024 to 29 March 2024.

2.1. Study Design and Database

The investigation was carried out following the planned scientific work of the Department of Microbiology, Virology, and Immunology of Poltava State Medical University (PSMU), Ukraine: "Study of the role of opportunistic and pathogenic infectious agents with different sensitivity to antimicrobial drugs in human pathology (No DR 0123 U102413)". The material from the fauces was taken from children with recurrent tonsillitis in the children's outpatient department of the Poltava Regional Clinical Hospital named after M.V. Sklifosovsky. The children were aged from five to eleven years (three girls aged 5, 6, and 8; two boys aged 6 and 11).

2.2. Methods of Investigation

The study was conducted in compliance with biosafety regulations [7]. The procedures were performed in accordance with the recommendations for basic laboratory procedures in clinical bacteriology [8]. Material was taken from the fauces with a sterile swab. The stamen probe was placed in Amies transport medium for transportation to the laboratory of the Microbiology, Virology and Immunology Department of PSMU. Columbia agar with 5% sheep blood (bioMarieux, France) was used for primary culture of microorganisms from patients with tonsillitis. The swabs were rubbed over one-quarter of a blood agar plate, and the rest of the plate was streaked with a sterile wire loop from quarter to quarter [8]. Colonies were streaked on Meat Peptone Agar (MPA) to obtain pure cultures. The cultures were grown for one to three days at 37 °C under aerobic conditions. Morphological and tinctorial properties were determined in the Gram-stained smears. Immersion microscopy was used. The final identification was performed by using the Vitec-2 compact bioMarieux automatic bacteriological analyser (France) according to the manufacturer's instructions with identification cards GP and GN. Cultures of *S. paucimobilis* were identified using the GN identification card. Pigmentation of *S. paucimobilis* was determined on Columbia agar.

Antimicrobial susceptibility was determined by inhibition zone diameters (EUCAST standardised disk diffusion methodology) on the Mueller-Hinton (MH) agar [9].

2.3. Data Analysis

To assess the colonisation of the fauces by *S. paucimobilis*, a semiquantitative method was used (rare, +, ++, or +++), where massive growth of bacteria with colonies over the entire surface of the plate is +++, growth in the first three quarters is ++, growth in the first two quarters is +, and growth in the first quarter only means rare [8]. Antimicrobial susceptibility was determined on Mueller-Hinton medium [6]. The size of the growth retardation zones was measured after 48 hours, given the slow growth of *S. paucimobilis*.

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The study followed the Helsinki Declaration on Ethical Principles of Medical Research Involving Human Subjects and was approved by the Commission on Biomedical Ethics of Poltava State Medical University (Minutes No 223 of 24.01.2024). At the beginning of the study, each patient signed an informed consent regarding the collection of material for the study, the steps of the study and the possible consequences.

3. Results

3.1. Frequency of Isolation and Assessment of Microbial Colonization

Among the bacteria that grew in the cultures from the fauces, in addition to *S. paucimobilis,* there were mainly *S. aureus*. *S. pyogenes* was not isolated.

Of the samples taken from children with tonsillitis (five children), *S. paucimobilis* was isolated in 60% of cases (three children). The growth of the general bacterial community was observed in the cultures of all examined sick children in three sectors (++). *S. paucimobilis,* when was present, was also observed in all three sectors (++). In the material from one child out of three, where *S. paucimobilis* was present, this species was the most abundant.

3.2. Properties of Isolated Cultures

Colonies of *S. paucimobilis* on Columbia agar appeared as small (0.5–1 mm), translucent (Figure 1).

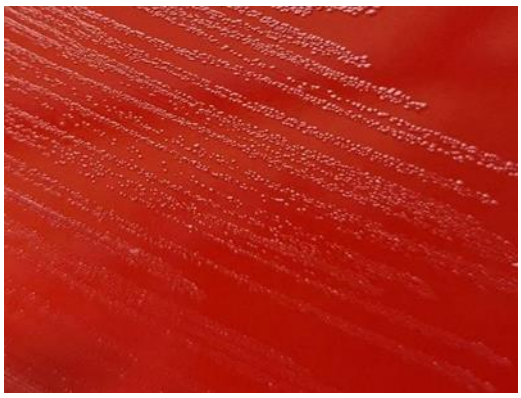

Figure 1. Growth of *S. paucimobilis* on blood agar.

The growth of *S. paucimobilis* was slower than that observed for other bacteria. Growth on MPA was slower than that observed on Columbia agar.

The intensity of pigmentation (yellow pigment) varied and was not pronounced in two isolates. More often, the colour of colonies appeared after several days of observation and was light yellow. Colonies of the third isolate had a brownish tint. The last one was characterised by slow α-haemolytic activity.

The growth pattern of colonies of different isolates during subsequent inoculations varied in terms of growth intensity, degree of transparency and strength of attachment to the medium. Re-seeding on MPA often proved to be problematic due to the subsequent slowdown in growth and the difficulty of sufficiently increasing the number of microorganisms for further research. During storage of cultures on MPA, the bacteria were closely adhered to the surface of the medium. The best results were obtained with blood agar streaks.

S. paucimobilis stained by Gram had the form of polymorphic Gram-negative rods, which were located singly, in pairs and, chains (Figure 2).

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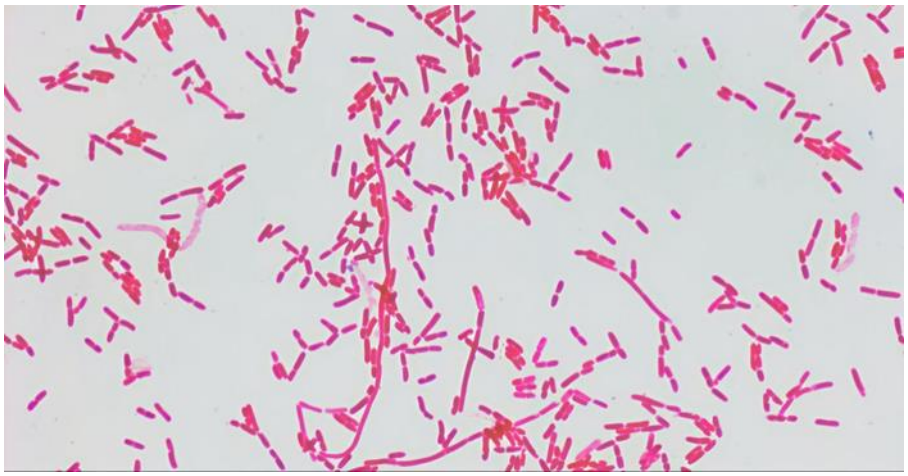

Figure 2. *S. paucimobilis* stained by Gram, immersion microscopy.

3.3. Sensitivity of Isolated Cultures to Antibiotics

There are no cards for determining the antimicrobial susceptibility of *S. paucimobilis* using the automated bacteriological analyser Vitek-2 compact bioMerieux (France). It is why antimicrobial susceptibility was determined on Mueller-Hinton medium [6] by measurement of the growth inhibition zone diameters (EUCAST standardised disk diffusion methodology) on the Mueller-Hinton (MH) agar [9]. The results of determining the sensitivity of bacteria to antibiotics are presented in Table 1.

Table 1. Antimicrobial susceptibility of *S. paucimobilis* isolates.

Antibiotic Tested	Diameter of growth retardation zones (mm)		
	Isolate No 1	Isolate No 2	Isolate No 3
Amoxicillin	30	20	21
Amoxicillin + Clavulanic acid	35	30	32
Ticarcillin + Clavulanic acid	35	29	30
Piperacillin Tazobactam	34	27	27
Ceftriaxone	38	29	23
Ceftazidime	25	19	18
Cefepime	28	23	25
Imipenem	36	22	29
Meropenem	38	20	25
Aztreonam	10	10	11
Tobramycin	40	32	18
Amikacin	19	21	16
Gentamicin	29	20	15
Cotrimoxazole	32	21	19
Rifampicin	25	25	25

The isolates showed varying susceptibility to different antibiotics, but in general, the bacteria were sensitive to most of them. The diameters of the growth inhibition zones often exceeded 20 mm, sometimes 30 mm. The diameter of the growth inhibition zone of isolate No 1 for Tobramycin was even 40 mm.

The diameters of the growth retardation zones were less than 20 mm for one or more isolates in the case of Ceftazidime, Amikacin, Gentamicin and the combination drug Cotrimoxazole.

The greatest variability in susceptibility was found for the combined drug Cotrimoxazole, when the diameters of the growth retardation zones differed for isolates 1 and 3 by 1.7 times. The cultures

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were the least sensitive to Aztreonam (the diameters of growth retardation zones were less than 11 mm).

4. Discussion

4.1. Background

The problem of infectious lesions of the human body by opportunistic microorganisms has been relevant for many decades. The list of such microorganisms includes primarily representatives of the genera *Staphylococcus, Streptococcus, Enterococcus, Escherichia, Candida*, and others [4–6], which can also be found in material taken from healthy people. *S. paucimobilis* occupies a special place, because it causes sporadic infections without being included in the list of microorganisms that are in any way associated with the body of a healthy person. Instead, it is known that *S. paucimobilis* are microorganisms of the natural environment, in particular soil and water [10].

4.2. Morphological, Tinctorial, Structural, and Biological Characteristics of S. paucimobilis

S. paucimobilis is an aerobic Gram-negative rod-shaped bacterium with unique characteristics of its outer membranes that distinguish it from many other Gram-negative bacteria. For example, instead of lipopolysaccharides, which are typical for Gram-negative bacteria, *S. paucimobilis* contains glycolipids rich in glucuronic acid and sphingolipids instead of lipid A. Some strains of *S. paucimobilis* can form a capsule or exopolysaccharides, which promote biofilm formation and survival in adverse conditions [1–3]. In our study, we investigated isolates identified as *S. paucimobilis*, which matched the morphological, tinctorial, and enzymatic properties reported in the literature. In smears stained by Gram and investigated under immersion microscope, bacteria of the isolates were located singly, in pairs, and chains. (Figure 2).

According to the literature, this microorganism often (but not always) has a yellow pigment, as it produces carotenoids [3]. In our studies of *S. paucimobilis* we observed two isolates with only slight yellowing cultures on MPA a few days after they were grown in a thermostat and then kept in a refrigerator at +4 °C. Exposure of cultures to natural light did not significantly affect pigment formation. One isolate out of three had a more intense colour of colonies (brownish colour). Both options are consistent with the literature [3].

4.3. Natural Sources of Infection Caused by S. paucimobilis as One of the Central Issues of Importance in Medical Practice

S. paucimobilis is rather a saprophyte, as it can be found in various environments: water (fresh and marine), soil, plants, and cave walls (several strains have been isolated from samples taken in Romanian caves) [14]. Thus, according to the generally accepted terminology, *S.paucimobilis* infection should be classified as a sapronotic infection by the source of infectious agent (Figure 3).

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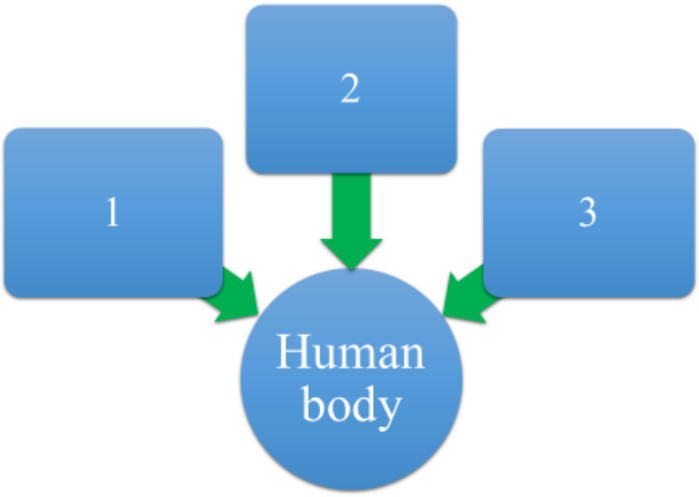

Figure 3. The classical idea of the sources of *S. paucimobilis.* 1. Natural environment (water, soil, etc.); 2. Objects of human habitation (elements of water supply systems, toys made of polymer materials, etc.); 3. Medical equipment, instruments, and drugs for parenteral administration.

4.4. Sources of S. paucimobilis in the Human Habitat in Hospital and Out-of-Hospital Environments

S. paucimobilis is resistant to disinfectants due to the presence of sphingolipids in the outer membrane and the formation of biofilms. This is important in the complex of measures to prevent the occurrence of hospital-acquired infections. Due to these features, it serves as an indicator of disinfection efficiency. Scientific observations of clinical cases focus on the source of infection, as clarity on this issue is the key in the prevention of new cases. However, very often the source of infection remains unclear [15].

It is known that *S. paucimobilis* can be present in hospital environments in dust particles, and can colonise various surfaces, equipment (ventilators, catheters, bronchofibroscopes), water supply systems, etc.) [16]. A case of isolation of *S. paucimobilis* from the surface of children's toys in a paediatric facility has been described [17]. The important characteristic feature of all these surfaces, which were found to be colonised by *S. paucimobilis* bacteria in hospitals, is the fact that they are surfaces of plastic products. Thus, the fact of *S. paucimobilis* getting on the mucous membranes of the human fauces of such ubiquitous microorganism should rather be considered as a regularity (Figure 3).

The high concentration of *S. paucimobilis* in pathological material from children with recurrent tonsillitis revealed in our study, and the high percentage of patients with this microorganism indicate a high probability that this microorganism can be transferred from patients to the environment (Figure 4).

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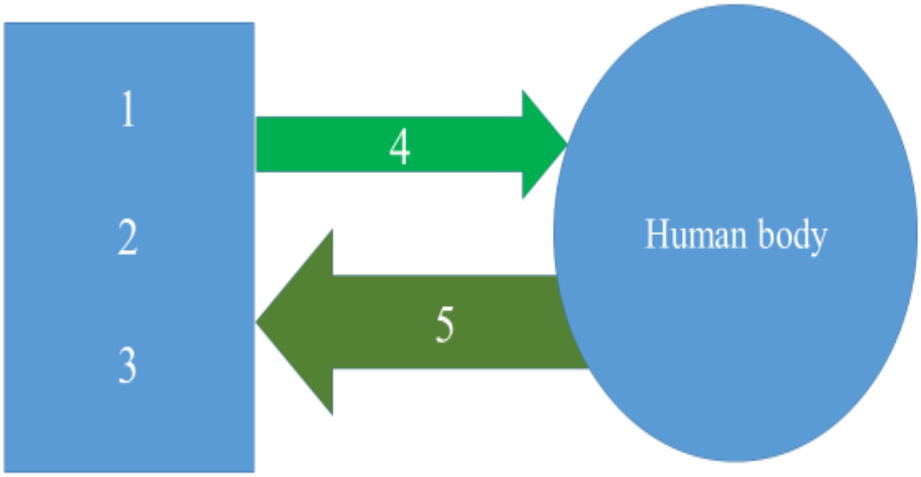

Figure 4. The updated idea of the sources of *S. paucimobilis* 1. Natural environment; 2. Objects of human habitation; 3. Medical equipment, instruments, and drugs for parenteral administration; 4. Classical imagination; 5. Additional information (in many cases of tonsillitis in children).

Therefore, a person can become infected not only through contact with environmental objects, but may also be the source from which *S. paucimobilis* enters the environment. This view is important for a more complete understanding of the specific epidemiology of clinical cases of infection caused *S. paucimobilis* when no other convincingly proven source has been identified, as is the case with hospital-acquired infections. Human beings, as well as environmental objects, are likely to be a source of *S. paucimobilis* in both hospital- and community-acquired infections.

4.5. Modes of Transmission in Hospital and Community-Acquired Exogenous Infection Caused by S. paucimobilis. Factors of Transmission

The routes of human infection with *S. paucimobilis* include iatrogenic, contact and airborne dust.

The literature describes clinical cases of bacteraemia caused by *S. paucimobilis* due to intravenous medication [18] and cases of peritonitis associated with continuous outpatient peritoneal dialysis [4]. In Korea, cases of co-infection with *S. paucimobilis* and *Chryseobacterium indologenes* have been reported for this mode of infection [19].

According to the literature, hospital-acquired infections caused by *S. paucimobilis* account for 69.0% of patients with bacteraemia. The majority of them were secondary infected in healthcare facilities as a result of the use of intravascular devices [5].

Cases of community-acquired infection caused by *S. paucimobilis* have also been described, where the source of infection was not identified [20]. Thus, it is generally accepted that sphingomonas infection is an exogenous sapronous infection by the source of infection in humans. The entry gate is blood, damaged skin, and mucous membranes.

Injectables, medical equipment, instruments and objects surrounding the person, as well as water from water supply systems, can transmit *S. paucimobilis*. Almost all reported clinical cases of hospital-acquired infections, including iatrogenic infections, include such objects as sources and transmission factors, including plastic.

The literature does not comprehensively describe from which source, through which transmission mechanisms, and due to which transmission factors a patient was infected when the

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infection was not associated with a hospital stay. As the isolates in our study were from mucosal samples of children treated as outpatients, the possibility of hospital infection did not seem likely. The children also did not attend the same childcare facility outside the hospital and did not belong to the same family, so there were no epidemiological links between individual patients. At the same time, our study found that *S. paucimobilis* was detected on the pharyngeal mucosa of children with recurrent tonsillitis in 60% of the cases studied. Thus, as in some cases described in the literature, in our investigation it is impossible to identify the source of infection of the examined children with *S. paucimobilis* bacteria.

4.6. Pathogenicity Factors

No significant pathogenicity factors of *S. paucimobilis* have been described in the literature. It is believed that the fundamentally different profile of enzymatic activity compared to other bacteria may be related to the pathogenesis of diseases caused by this microorganism [4].

The ability to biofilm formation, which was not determined experimentally in our investigation, but manifested as extremely strong adhesion to the surface of MPA when cultures were stored at +4 °C, may also play a role *in vivo*. Our observation is consistent with literature data regarding the pronounced adhesive activity of *S. paucimobilis*, which is provided by exopolymers of this microorganism [20].

The low immunogenicity of *S. paucimobilis* is determined by the peculiarities of the chemical composition of its outer membranes [1–3].

One of our isolates on Columbia agar was characterised by slow α-haemolytic activity. We have not found any information in the literature on the presence of toxins such as haemolysin in *S. paucimobilis* isolates. We assume that the haemolytic effect of some isolates we found may be the result of the complex action of a wide range of enzymes characteristic of this bacterial species.

No deaths have been reported in the literature related to *S. paucimobilis*. However, it is likely that *S. paucimobilis* is a more important pathogen than previously thought [21].

4.7. A susceptible Organism

Knowledge of the role of *S. paucimobilis* as an infectious agent is limited, as it is rarely isolated from human pathological material [16–19]. The development of generalised or localised infections caused by it and not associated with iatrogenic intervention is considered more likely due to the inability of the immune system to fully protect the body, which arose due to one reason or another, to counteract even a practically non-pathogenic microorganism [19].

An outbreak caused by *S. paucimobilis* in a paediatric haematological and oncological hospital was described, when haemoculture of the pathogen was obtained from samples from 51 patients over a two-year period, when the microorganism was also isolated from water samples from the hot water supply system in this institution [23]. A case of meningitis caused by *S. paucimobilis* and *M. tuberculosis* in an immunocompromised patient was also described [24].

However, in some clinical cases, an atypical infection caused by *S. paucimobilis* has been described, when, for example, a 59-year-old immunocompetent patient had a retropharyngeal abscess, and *S. paucimobilis* was isolated as a haemoculture [25]. A case of sphingomonas infection of the eye has also been described [20]. Thus, *S. paucimobilis* can affect both healthy people and people with immunodeficiency. Although this microorganism has a low level of virulence and no deaths have been reported in patients with sphingomonas paucimobilis infection [26], it can cause septic shock [4]. The frequent isolation of *S. paucimobilis* in our study may be due to both the age-related immune system of children and the fact that these were children with recurrent infection, which in itself is a sign of a decrease in the immune defence.

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4.8. Etiotropic Therapy

It is reported in the literature that *S. paucimobilis* is usually susceptible to aminoglycosides, fluoroquinolones, aminoglycosides, trimethoprim sulfamethoxazole, and some third-generation Cephalosporins [1]. In our study, the diameters of growth retardation zones for β-lactam antibiotics were ≥20 mm, and for clavulanic acid-protected antibiotics, this figure was much higher (from 29 to 35 mm for different isolates) and highly variable for different isolates. In addition, the diameters of bacterial growth inhibition were larger for Piperacillin Tazobactam compared to Amoxicillin.

Among the Cephalosporins, Ceftazidime was less effective, with zone diameters of 19 and 18 mm for 2 isolates, respectively. For Ceftriaxone and Cefepime, this figure was at least 23 mm. Thus, the results are consistent with the data reported in the literature [1]. The results for the Carbopenems varied widely for different isolates – from 20 to 38 mm for Meropenem and from 22 to 36 mm for Imipenem.

The diameters of the zones of no growth for aminoglycosides varied in a wide range – from 15 to 40 mm, depending on the preparation and an isolate. That is, in some cases, this is consistent with the literature, but we found variability in these indicators depending on the isolate.

For Cotrimoxazole, the value ranged from 19 to 32 mm, and for Rifampicin, the value was constant for all isolates (25 mm).

Aztreonam was the least effective, despite the fact that it is considered to be effective in treating infections caused by Gram-negative bacteria such as *P. aeruginosa*. The reasons for this phenomenon should be sought in the peculiarities of the *S. paucimobilis* chemical structure and metabolic processes.

Thus, our results of the study of antibiotic susceptibility of isolates indicate a sufficient level of susceptibility of *S. paucimobilis* to many antimicrobial agents, as reported in the literature [1,2], but also the possible variability of these traits, and, therefore, the possibility of the emergence of more resistant forms. Therefore, the controlled use of antimicrobials is as relevant for the control of *S. paucimobilis* as for diseases of other microbial aetiology. According to the results of this study, the clavulanic acid-protected β-lactam antibiotics, Piperacillin Tazobactam and Carbopenems, and in cases of resistance to other antibiotics, the anti-TB antibiotic Rifampicin, are the best drugs of choice for the empirical antibiotic therapy.

5. Conclusions

1. The obtained results do not confirm or refute the role of *S. paucimobilis* in microbial associations in the development of infectious pathology of the fauces of children with recurrent tonsillitis. However, it indicates that the presence of this species of microorganisms in the microbial landscape of the pharyngeal mucosa of children with recurrent tonsillitis is a common phenomenon, which, in particular, can be explained by age-related immune system in children.
2. The high concentration of *S. paucimobilis* in pathological material from children with recurrent tonsillitis and the high percentage of patients with this microorganism indicate a high probability that this microorganism can be transferred from patients to the environment.
3. Among the antimicrobials of choice for empirical antibiotic therapy, the best ones are the clavulanic acid-protected β-lactam antibiotics, Piperacillin Tazobactam, and Carbopenems. The anti-TB antibiotic Rifampicin is useful in cases of resistance to other antibiotics.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the **Commission on Biomedical Ethics of Poltava State Medical University, Ukraine (Minutes No. 223 of 24.01.2024).**

Informed Consent Statement: Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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